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A METHOD FOR DETERMINATION OF PARTICLES OF BROWN TO BLACK SEED  
COAT AND SCORCHED AND DISCOLORED PEANUT TISSUE IN PEANUT BUTTER  
(Issued September 1, 1942)

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Recommended Equipment

Strong case knife or 8 inch to 10 inch spatula  
Scalpel or small spatula with tapered end  
Analytical balance  
Weighing dish and counterpoise or counterpoise for 55 cc. glass-stoppered specific gravity bottles  
Test tubes, 6 inches long by 1 inch in diameter, fitted with No. 4 rubber stoppers, or 55 cc. (approximately) glass-stoppered specific gravity bottles  
400 to 600 cc. beaker  
Vacuum filter  
Buchner funnel fitted with center bored rubber stopper to fit into a two-liter suction flask  
Two-liter suction flask  
Normal Sodium Hydroxide (NaOH) Solution  
1/2 Normal Sodium Hydroxide (NaOH) Solution  
Water - tap or distilled  
Filter paper (coarse, rapid filtering)  
Petri dishes  
Dissecting needle  
Small curved tweezers  
Wide-field, Greenough-type binocular microscope fitted with 10x eyepieces and 2.0x objective. One of the eyepieces should be equipped with a micrometer disc engraved with a square formed by two sets of parallel lines to represent an area of 0.25 mm. x 0.25 mm. (1/4 mm. x 1/4 mm.) in size on the object plane

Procedure

Mix the peanut butter sample thoroughly with a strong case knife or spatula. If a determination is to be made of a composite sample, mix contents of each jar thoroughly, take 100 grams of peanut butter from each jar, place in a beaker, and mix the composite sample thoroughly.

Use a scalpel or small spatula to handle the sample to be tested and weigh one gram of the well mixed sample into a 6-inch test tube or a 55 cc. glass-stoppered specific gravity bottle.

Fill the test tube or bottle one-third full of Normal Sodium Hydroxide solution, close with stopper and shake vigorously until the peanut butter disintegrates. Allow the contents to settle until saponification has evidently been completed and all particles of seed coat have settled to the bottom of the test tube or bottle. Decant the liquid carefully and discontinue decantation before the particles of seed coat rise to flow off.

Fill the test tube approximately half full of 1/2 Normal Sodium Hydroxide solution, shake well, and allow contents to settle again until saponification has been completed. Decant again.

Fill the test tube approximately half full of hot water and shake again to wash out the hydroxide. Allow contents to settle and decant for the third time.

Add enough water to wash down the particles of seed coat and wash out into a coarse, rapid filtering filter paper placed in the Buchner funnel. The filter paper should be larger than the inside dimension of the Buchner funnel and should have been previously ruled with parallel lines (about 1 cm. apart) or a ring strung with fine parallel wires 1 cm. apart may be used as guides in counting the particles of seed coat. A lead pencil should be used to line the filter paper as hydroxide removes ink.

Filtration will be hastened if a copper screen of about 80 mesh and 5.5 cm. in diameter is placed under the filter paper.

After the filtering process has been completed, remove the filter paper carefully with crooked tweezers to a shallow container or plate that can be moved about under the microscope. A 100 mm. Petri dish is ideal for this purpose. Place the carrier containing the particles under the microscope and move to right and left, using the lines as guides to avoid overlapping and duplicating counts made.

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Particles that are equal to, or are larger than, one-half the area of a 1/4 mm. square (or 1/32 of a sq. mm.) are counted; particles that are less than this area in size are disregarded. Particles counted should include not only seed coat but scorched cellular tissue of the peanut or any other particles that cause a noticeable dark speck. The total number of particles of seed coat and scorched or discolored peanut tissue counted is considered the "particle count per gram" for the sample examined.

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If a clinical centrifuge is available, the particles of seed coat, scorched or discolored peanut tissue may be determined in accordance with the following method:

Use a scalpel or small spatula to handle the sample to be tested and weigh one gram of the well-mixed sample as prepared in the foregoing method into a 50 cc. centrifuge tube. Fill the tube about one-half full of approximately normal Sodium Hydroxide solution, stopper the tube and shake until the peanut butter disintegrates. Place the tube in a centrifuge which has been properly counterbalanced and centrifuge for about three to five minutes at a moderate speed.

After centrifuging, decant the supernatant liquid and repeat the procedure, once with sodium hydroxide solution and twice with hot water. Then wash the centrifuge upon a coarse, rapid filtering filter paper, ruled to facilitate counting, placed in a suction filter.

After filtering, count the particles as outlined in the foregoing method.